hydrogen phosphate solution/methanol followed by acetonitrile/water. Although OPA is used as the derivatization reagent by the majority of laboratories, other reagents have been employed including naphthalendialdehyde, fluoronitrobenzofurazan and fluorescamine. The last reagent is unsatisfactory as it generates two peaks in the HPLC chromatogram for fumonisin B_1.

Three recently developed methods for the determination of the fumonisins in corn-based commodities are outlined in **Table 13**. Method 1 uses a combination of SAX and C_{18} SPE clean-up prior to ion-pair HPLC and fluorescence detection; on-line derivatization within a reaction coil is employed. The recovery of the fumonisins ranged from 54 to 110% at 40 and 80 $\mu g\,kg^{-1}$, respectively. Method 2 is an automated procedure using on-line immunoaffinity clean-up, reversed-phase HPLC and electrospray ionization MS detection. The protonated molecule signal (m/z 722) was used to achieve a limit of quantification of 250 pg.

An HPTLC method (Method 3, **Table 13**), for the determination of fumonisin B_1 in rice, has recently been reported. A novel derivatization step involved the brief immersion of the HPTLC plate in a 0.16% acidic solution of *p*-anisaldehyde, followed by quantification by scanning fluorodensitometry. The response was linear over the range 0 to 5 $mg\,kg^{-1}$ (ppm).

An intercomparison study on a variety of methods for the determination of the fumonisins in maize has recently been undertaken under the auspices of the European Commission, Measurements and Testing Programme. Twenty-four laboratories participated, using their normal routine procedure for the determination of fumonisins B_1 and B_2 in the range 0.5–3.0 and 0.2–1.5 $mg\,kg^{-1}$ (ppm), respectively. All laboratories used a similar method involving extraction with methanol/water, clean-up with an SAX SPE column and HPLC fluorescence quantification of the OPA derivative. The intra- and interlaboratory precisions were high (10 and 11%, respectively, for fumonisin B_1; and 11 and 13%, respectively, for fumonisin B_2). However, the recoveries were low ($70 \pm 14\%$ and $69 \pm 16\%$ for fumonisins B_1 and B_2, respectively). Interestingly, higher recoveries were associated with extraction by shaking ($85 \pm 12\%$ for fumonisin B_1) rather than by blending ($62 \pm 6\%$).

Conclusions

The continued use of a variety of chromatographic procedures for the determination of mycotoxins is envisaged. Although HPLC is the method of choice in the developed world for a wide range of applications, it is important that precise and accurate methods continue to be developed that are appropriate to the special needs of developing country laboratories.

See Colour Plate 53.

See also: **II/Affinity Separation:** Immunoaffinity Chromatography. **Chromatography: Gas:** Detectors: Mass Spectrometry. **Chromatography: Liquid:** Derivatization. **III/Aflatoxins and Mycotoxins:** Thin Layer (Planar) Chromatography. **Membrane Preparation:** Phase Inversion Membranes.

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Thin-Layer (Planar) Chromatography

M. E. Stack, US Food and Drug Administration, Washington DC, USA

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The aflatoxins are toxic and carcinogenic metabolites of the moulds *Aspergillus flavus* and *A. parasiticus*. They are often found as contaminants of peanuts, tree nuts, corn and cottonseed. They were discovered as a result of investigations into Turkey X disease in Britain, in which 100 000 turkeys and numerous other poultry died as a result of feeding on peanut meal which had been contaminated with mould.

Thin-layer chromatography (TLC) played a crucial part in the discovery and subsequent research on the aflatoxins and continues to play an important part in the analytical methods used for control of aflatoxins in food and feeds. The four major compounds are aflatoxins B_1, B_2, G_1 and G_2. Aflatoxins B_1 and B_2 have bright blue fluorescence on TLC and G_1 and G_2 are bright green-blue. Aflatoxin B_1 is found in the largest amounts in samples and is also the most toxic and carcinogenic of the four. Aflatoxin M_1 is found in the milk of animals which have ingested aflatoxin B_1.

For the structures of the aflatoxins see **Figure 1**. After the discovery of the aflatoxins other mycotoxins were discovered and methods of analysis using TLC have been devised for them.

Preparation of Samples

Aflatoxin contamination of food and feeds is usually in the range of ng g^{-1} to μg g^{-1}. Sampling error is a severe problem in aflatoxin determination because only a few affected kernels can contaminate a large amount of finished product. Amounts as high as 207 000 ng g^{-1} have been found in individual corn kernels. This is sufficient aflatoxin to produce a level of contamination of 20 ng g^{-1} in a batch of 10 000 kernels of grain. Sampling plans have been developed for various commodities. In general, the larger the unit size of the commodity, the larger the sample size should be. The sample should be finely ground and mixed before taking out the analytical test portion. Often the sampling error is larger than the analytical error.

Various methods of analysis have been devised. Many of these have been published in the *Official Methods of Analysis of AOAC International*, after collaborative studies by several laboratories. If the precision and accuracy of the results are acceptable the method becomes official.

The three most widely used extraction and clean-up methods for preparing aflatoxin extracts for TLC are the CB method, the BF method and the immunoaffinity column method. The CB method, named after the Contaminants Branch of the US Food and Drug Administration (FDA), uses chloroform extraction, filtering through paper, addition to a silica gel column, washing with hexane and ether, elution with chloroform–methanol (97 : 3 v/v), and evaporation to dryness to prepare the extract for TLC. The BF method, named after the Best Foods Company, uses methanol–water (55 : 45 v/v) extraction, hexane

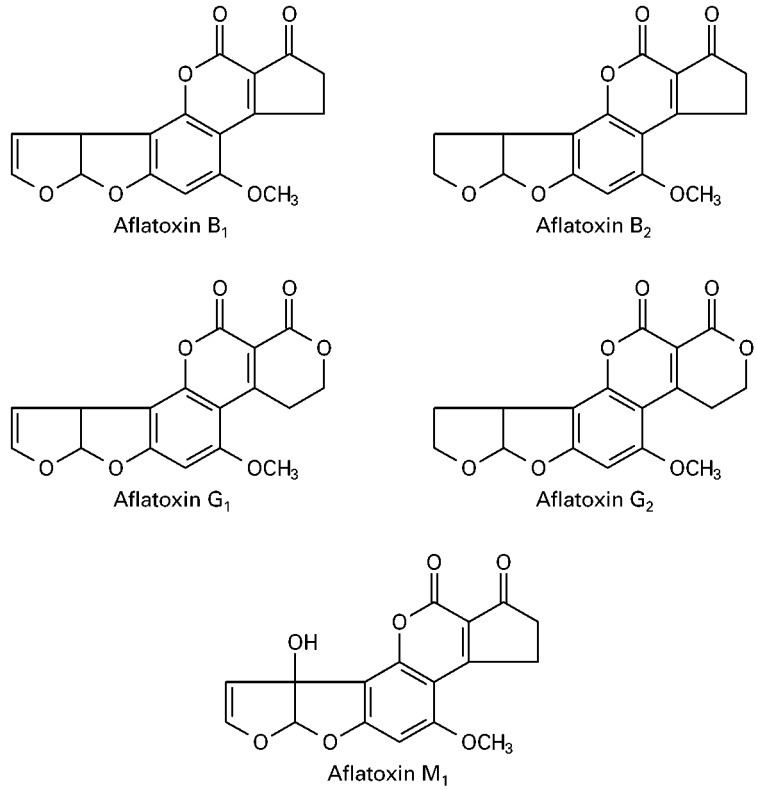

Figure 1 Structures of aflatoxins B_1, B_2, G_1, G_2 and M_1.

defatting in a separatory funnel, partition into chloroform and evaporation to dryness to prepare the extract for TLC. The immunoaffinity column method uses methanol–water (7 : 3 v/v) for extraction, filtering through paper, dilution with water, filtering through a glass microfibre filter, application to a column upon which antibodies to aflatoxins have been bound, washing with water, elution with methanol and evaporation to dryness to prepare the extract for TLC.

The advantage of the CB method is that it is precise and accurate when correctly performed. Disadvantages include the acquisition and disposal costs of the reagents used. The advantage of the BF method is that it has the lowest cost of any of the methods. The disadvantage is that it results in a somewhat dirtier extract. The advantages of the immunoaffinity column method are its simplicity of performance and the purity of the aflatoxins in the extract. Its disadvantage is the high cost of the columns.

After evaporation, in all three methods, the extract is carefully transferred using rinses of chloroform to a small vial. The solvent is again evaporated to dryness in a water bath under a stream of nitrogen. The residue is dissolved in a small amount of solvent (200 μL), usually benzene–acetonitrile (98 : 2 v/v), for spotting on TLC. Since the use of benzene is sometimes prohibited because of its toxicity, other solvents such as toluene–acetonitrile (9 : 1 v/v) may be used as well.

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Aflatoxin Standards

A large source of error in the analysis is due to incorrectly prepared aflatoxin standards. In a check sample series, standards were found that contained more or less than the stated amount of aflatoxins when compared with a correctly prepared standard sent out with the study. It is very important to work with pure and accurate standards if accurate quantitative and qualitative results are to be obtained. Aflatoxin standards may be purchased from chemical distributors but need to be checked by means of TLC or liquid chromatography (LC) to ensure that they are pure. Small quantities of aflatoxin standards are sometimes available from organizations such as the FDA without cost.

Crystalline aflatoxin standards should be handled in a glove box because of their carcinogenicity and the electrostatic nature of the crystals. Because of the difficulty in handling the crystalline material, the aflatoxins are often received as dry films deposited in a precise amount in the bottom of a glass vial. The contents of the vial should be dissolved in the solvent (benzene–acetonitrile, 98 : 2 v/v) and mixed on a vortex mixer for 1 min since the standards do not dissolve rapidly. After mixing, the solution is transferred to a screw-cap vial and the ultraviolet spectra measured between 370 and 330 nm. The concentration can then be calculated using the values listed in the AOAC International Official Method 971.22. The standards are applied to a TLC plate to verify the purity. The solutions must be stored in a closed and sealed vial in a refrigerator at 4–8°C. Mixtures of the four major aflatoxins can be prepared by diluting the concentrated stock solutions. The mixture most often used contains aflatoxins B_1 and G_1 at 1.0 μg mL^{-1} and B_2 and G_2 at 0.2 μg mL^{-1}. This ratio between the four aflatoxins approximates to the ratio found in some sample extracts. The aflatoxins in benzene–acetonitrile (98 : 2 v/v) are stable when stored in a closed and sealed vial in a refrigerator at 4–8°C. Evaporation or decomposition of the aflatoxins can be detected using TLC or LC, shown by additional spots or additional peaks or an increase or decrease in the fluorescent intensity, as evidenced by unusual area integration values from the LC detector or TLC densitometer.

Spotting, Development and Examination of the TLC Plate

The plates most often used for aflatoxin analyses are 20 × 20 cm glass plates, pre-coated with a 0.25 mm layer of silica gel 60 (E. Merck, Darmstadt); plates from other manufacturers may work equally well. Spotting should be done in subdued incandescent light to avoid photodecomposition of the aflatoxins. Using a 10 μL syringe, on an imaginary line 4 cm from the bottom of the plate and 1 cm apart, 2, 5 and two 10 μL spots of the sample extract are applied together with 2, 5 and 10 μL spots of mixed aflatoxin standards; 5 μL of the standard is applied on top of one of the 10 μL spots of sample extract. It is possible to spot four samples on to each plate.

The plate is developed for less than 90 min with acetone–chloroform (1 : 9 v/v) until solvent is within 4 cm of the top of the plate. It may be necessary to adjust the acetone–chloroform ratio to obtain optimum resolution. The plate is removed from the tank and air-dried in the hood in the dark.

Plates are examined under long wave ultraviolet light at 365 nm in a cabinet equipped with a filter for protecting the eyes from the ultraviolet light. Aflatoxins appear in order of decreasing R_F: B_1, B_2, G_1 and G_2. G_1 and G_2 are slightly greener than the blue B_1 and B_2. The R_F values for the aflatoxins in the sample spots should be the same as those of the standard spots. The aflatoxins in the sample spot upon which the standard is superimposed should

coincide exactly with the standard spots. The intensity of the fluorescence of each of the sample spots may be compared with that of the standard spots to estimate the amount of aflatoxin present in the extract. Separate estimates need to be made for B_1, B_2, G_1 and G_2. If the spots of the smallest portion of the sample are more fluorescent than the strongest standard spot it is necessary to dilute the sample extract and re-chromatograph. The plate may be run on a densitometer equipped with an ultraviolet light source set at 365 nm and an ultraviolet filter before the photomultiplier detector. Connecting the TLC densitometer to a computer permits the integration, calculation, printing, and storage of results. If more accurate quantitative results are necessary, the extract can be re-diluted to a concentration approximately equal to that of the standard and re-chromatographed in the same manner as above. The concentration of each aflatoxin in the extract can be calculated using the formula:

$$\text{ng g}^{-1} = (S \times Y \times V)/(X \times W)$$

where $S = \mu$L of standard spot equal to sample; $Y =$ concentration of standard in ng μL^{-1}; $V = \mu$L of final dilution of sample extract; $X = \mu$L of sample spot equal to standard; and $W =$ grams of sample that the extract represents.

Not all blue fluorescent spots in the extracts are necessarily aflatoxins. Sample extracts may contain interferences, especially at the R_F values of G_1 and G_2. Respotting with an alternative solvent system such as the top phase benzene–ethyl alcohol–water (46 : 35 : 19 v/v) or with benzene–methanol–acetic acid (90 : 5 : 5 v/v) often resolves the aflatoxins from the interferences. Other solvents which are sometimes used are: ether–methanol–water (96 : 3 : 1 v/v), chloroform–acetone–water (88 : 12 : 1.5 v/v), or chloroform–acetone–isopropanol–water (88 : 12 : 1.5 : 1 v/v).

Two-Dimensional TLC

Another powerful technique for resolving the aflatoxins from interferences is two-dimensional TLC. In this technique two spots of aflatoxin standards and one spot of sample extract are spotted on the plate, as shown in **Figure 2**. The plate is first developed with ethyl ether–methanol–water (96 : 3 : 1 v/v) in the first direction. After development and air drying, the plate is redeveloped in the second direction with acetone–chloroform (1 : 9 v/v). After development and air drying the plate is examined under ultraviolet light at 365 nm for aflatoxin spots. A blue spot should appear at the intersection of imaginary lines from the two standard spots. The two-dimensional technique works well for difficult materials such as eggs and spices.

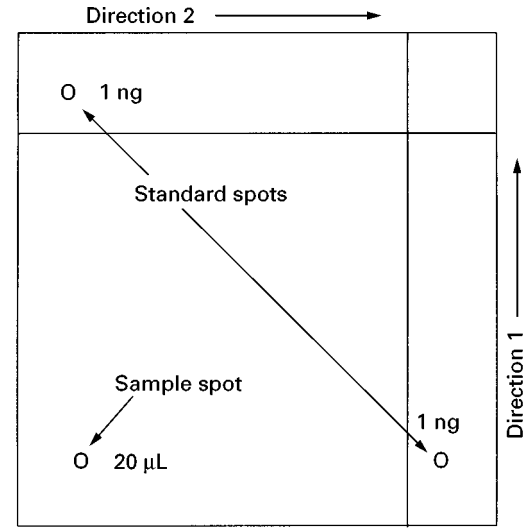

Figure 2 Two-dimensional TLC plate for aflatoxin analysis.

Confirmation of Identity of Aflatoxins

To confirm the identities of aflatoxins B_1 and G_1 a technique has been devised which uses derivative formation on the TLC plate. The sample extracts and standards are spotted on the origin line of the plate and 1 μL amounts of trifluoroacetic acid are then added to each spot. After reacting for 5 min, the trifluoroacetic acid is removed by blowing air at 35–40°C on the plate for 10 min. The trifluoroacetic acid catalyses the addition of water across the double bond in the terminal furan ring of aflatoxins B_1 and G_1 to form the derivatives called aflatoxin B_{2a} and G_{2a}, which give lower R_F values than the parent compounds. The plate is developed with chloroform–acetone (85 : 15 v/v). Upon examination of the plate under ultraviolet light at 365 nm, sample and standard will have low R_F blue and green spots of the derivatized aflatoxins. Since aflatoxin B_2 and G_2 do not have the unsaturated double bond, they will be unaffected by the test and will appear at their normal R_F values. For additional confirmation the plates can be sprayed with sulfuric acid–water (1 : 3 v/v), which causes the aflatoxin spots to change from blue or blue-green to yellow fluorescence.

Mass Spectrometric Confirmation of the Aflatoxins

The aflatoxins may be confirmed by negative ion chemical ionization–mass spectrometry. The aflatoxin is first purified using preparative TLC. The entire extract is applied along the origin line of a TLC

plate which is developed using chloroform–acetone (9 : 1 v/v). After drying, the silica gel is scraped from a band containing the aflatoxin B_1. If the silica gel is scraped into a sintered glass funnel the aflatoxin can be eluted with chloroform–methanol (2 : 1 v/v). After evaporation and re-dissolving in acetone, the aflatoxin can be introduced into the inlet probe of the mass spectrometer and spectra of sample and standard aflatoxin compared.

Methods of Analysis for Aflatoxin M_1

When cows consume aflatoxin in their feed, a small percentage of it is metabolized and excreted in the milk in the form of aflatoxin M_1. Aflatoxin M_1 is also toxic and carcinogenic, so methods have been developed to detect it in milk. Since infants and children are major consumers of milk products, the levels of concern for M_1 in milk are set quite low by various countries, in the range of 0.05–0.5 $\mu g\,L^{-1}$. Analyses of milk and cheese samples at these low levels are more difficult. One method of analysis uses partition from the milk into chloroform and silica gel column clean-up before the TLC determination. Another method used extraction from the milk on to a C_{18} solid-phase extraction column and clean-up on a silica gel column before TLC or LC determination. An immunoaffinity column clean-up can also be used.

TLC is accomplished on 10×10 cm or 20×20 cm, 0.25 mm layer thickness silica gel 60 plates developed with chloroform–acetone–isopropanol (87 : 10 : 3 v/v). Other solvent systems which have been used are ether–methanol–water (95 : 4 : 1 v/v) and ether–hexane–methanol–water (87 : 10 : 4 : 1 v/v).

A two-dimensional TLC method for aflatoxin M_1 has been developed for liver but also works for milk and cheese extracts. The plate is spotted in a similar manner to the two-dimensional plate for aflatoxin B_1 and developed in the first direction with ether–methanol–water (95 : 4 : 1 v/v) and after development and drying is developed in the second direction with chloroform–acetone–isopropanol (87 : 10 : 3 v/v). The developed plate is examined under ultraviolet light at 365 nm for a blue spot at the intersection of imaginary lines from the two standard spots.

The confirmatory technique using trifluoroacetic acid works for aflatoxin M_1 as well but is performed using two-dimensional TLC and requires heating the plate in an oven at 75°C for the reaction to occur.

TLC Determination of Other Mycotoxins

Mycotoxins can be generated by a large number of mould species. Several books review the incidence and toxicity of the most common mycotoxins. The interest of the regulatory authorities has been focused on relatively few of these metabolites that cause problems in human and animal health. The mycotoxins of regulatory interest are currently the aflatoxins, ochratoxin A, patulin, fumonisins, deoxynivalenol, other trichothecenes and zearalenone. TLC procedures are described below for these mycotoxins.

Ochratoxin A

Ochratoxin A (**Figure 3**) is a metabolite of some *Aspergillus* and *Penicillium* species. It is found as a contaminant of barley, corn, wheat, oats and coffee. It has also been found in meat, human blood and human milk. Ochratoxin A causes porcine nephropathy, notably in some Scandinavian countries when contaminated barley is fed to swine. Ochratoxin A is extracted from samples with chloroform in the presence of phosphoric acid and cleaned up using partition into sodium bicarbonate and C_{18} solid-phase extraction. In a similar manner to the TLC of aflatoxin discussed above, ochratoxin A is spotted on a plate pre-coated with a 0.25 mm layer of silica gel 60 (E. Merck, Darmstadt) and developed with benzene–methanol–acetic acid (18 : 1 : 1 v/v) or toluene–ethyl acetate–formic acid (5 : 4 : 1 v/v). After drying, the plate is examined under long and short wave ultraviolet light (365 and 254 nm). Ochratoxin A ($R_F = 0.65$) fluoresces brightest under long wave ultraviolet light and is usually accompanied by the less toxic ochratoxin B ($R_F = 0.5$) which fluoresces brightest under short wave ultraviolet light. The fluorescence of the ochratoxins can be enhanced by spraying the plate with alcoholic sodium bicarbonate solution which changes them to their more fluorescent salt forms. The ochratoxins can be confirmed by ester formation using boron trifluoride in ethanol and re-chromatographing using the same conditions as above. The ethyl esters appear at lower R_F values than the parent compounds under long and short wave ultraviolet light.

Patulin

Patulin (Figure 3) is a lactone metabolite of several moulds, including *Penicillium expansum*, which causes brown rot in apples. Patulin is often found in apple juice, especially juice from fallen apples. Patulin can be extracted from apple juice with ethyl acetate and cleaned up using silica gel column chromatography. After evaporation, the extract is dissolved in chloroform and spotted on silica gel plates and developed with toluene–ethyl acetate–formic acid (5 : 4 : 1 v/v). After drying, the plate is sprayed

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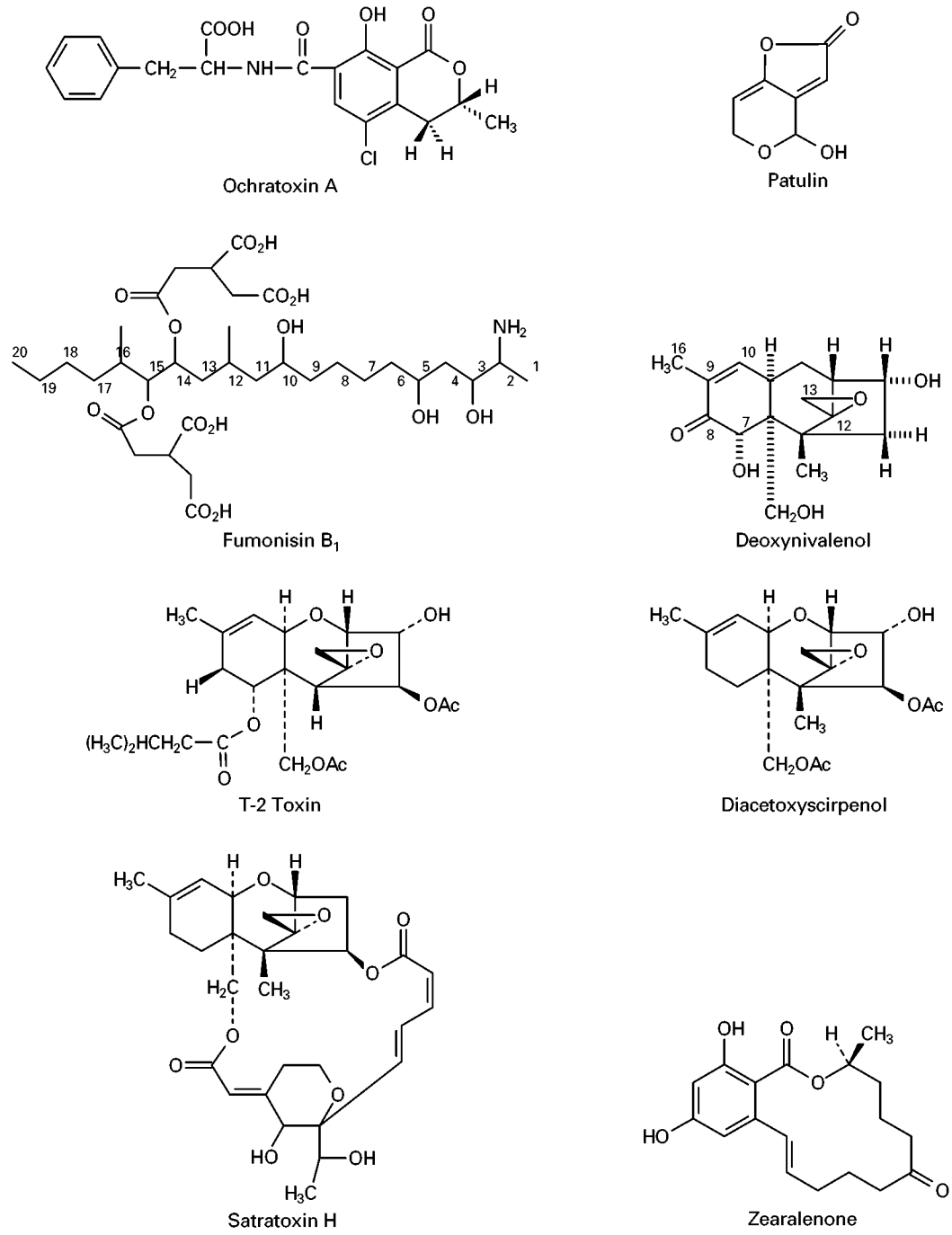

Figure 3 Structures of ochratoxin A, patulin, fumonisin B_1, deoxynivalenol, T-2 toxin, diacetoxyscirpenol, satratoxin H and zearalenone.

with 3-methyl-2-benzothiazolinone hydrazone–HCl (MBTH) solution and heated for 15 min in an oven at 130°. Under ultraviolet light at 365 nm, patulin ($R_F = 0.5$) appears as a yellow-brown fluorescent spot. The amount of patulin in the sample can be determined by comparing the intensity of fluorescence of the standard and sample spots. Other TLC developers, such as hexane–anhydrous ether (1 : 3 v/v), chloroform–methanol (95 : 5 v/v), and chloroform–acetone (9 : 1 v/v) can be used to confirm the identity of the patulin. After development, plates are sprayed with MBTH to reveal the patulin.

Fumonisins

Fumonisins B_1 (**Figure 2**) and B_2 are metabolites of *Fusarium moniliforme* and *F. proliferatum*. They are common natural contaminants of corn and have

caused deaths in horses and swine. Small amounts have been found in cornmeal and breakfast cereals. In order to ensure that they are not present in food in excessive amounts, methods of analysis have been developed. Most methods use LC after formation of derivatives of the primary amine function with reagents such as *o*-phthaldialdehyde. However, a reversed-phase TLC determination has been devised. The fumonisins, dissolved in acetonitrile–water (1 : 1 v/v), are spotted at the origin of a 10×10 cm C_{18} plate and developed with methanol–1% KCl in water (3 : 1 v/v). After drying, the plates are sprayed with 0.1 mol L^{-1} sodium borate (pH 8–9) followed by fluorescamine (0.4 mg mL^{-1} in acetonitrile). After a 1 min delay, further spraying with 0.01 mol L^{-1} boric acid–acetonitrile (2 : 3 v/v) is carried out. Examination under 365 nm ultraviolet light reveals fluorescent yellow spots of fumonisin B_1 ($R_F = 0.5$) and fumonisin B_2 ($R_F = 0.1$).

Deoxynivalenol

Deoxynivalenol (**Figure 2**), also called vomitoxin, is a trichothecene metabolite of *F. graminearum*, an organism which causes a disease in barley and wheat called head blight or scab. Deoxynivalenol is found as a contaminant of barley, wheat, corn and rye and causes adverse health effects in animals and humans, including feed refusal and vomiting in swine. An advisory level of 1 μg g^{-1} has been set for finished wheat products. Methods for analysis have been devised using LC and TLC. The TLC method uses acetonitrile–water (84 : 16 v/v) extraction and cleanup using a charcoal–alumina–Celite (7 : 5 : 3 v/v) column. The extracts and standard deoxynivalenol are dissolved in methanol and spotted near the 20 cm edge of a 20×10 cm Linear-K High Performance (Whatman, Clifton NJ) or equivalent silica gel plate and developed with chloroform–acetone–2-propanol (8 : 1 : 1 v/v). After drying the plate is sprayed with aluminium chloride solution (20 g $AlCl_3.6H_2O$ in 100 mL methanol–water: 1 : 1 v/v) and then heated in an oven at 120° for 7 min. Under 365 nm ultraviolet light, deoxynivalenol appears as a blue fluorescent spot at $R_F = 0.78$. Spots may be scanned with a densitometer.

Other Trichothecenes

The trichothecenes are a large group of fungal metabolites produced by various species of *Fusarium*, *Myrothecium*, *Stachybotrys*, *Verticimonosporium*, *Cylindrocarpon*, *Trichoderma* and *Tricothecium*. They have been implicated in numerous farm-animal poisonings. A human disease called alimentary toxic aleukia occurred in the former Soviet Union during World War II when grains were eaten after they had lain out in the field under snow during the winter. *Fusarium* moulds isolated from these grains were shown to produce large amounts of T-2 toxin (Figure 3) and related derivatives. T-2 toxin and related compounds can be analysed by silica gel TLC using chloroform–methanol (9 : 1 v/v) as the developer. Since the trichothecenes are colourless and do not fluoresce, it is necessary to spray the developed plate with sulfuric acid–methanol (1 : 1 v/v), heat for 10 min at 100°C and examine the plate under 365 nm ultraviolet light. Trichothecenes of the T-2 group will appear as blue fluorescent spots, T-2 at $R_F = 0.64$, diacetoxyscirpenol (Figure 3) at $R_F = 0.60$, neosolaniol at $R_F = 0.39$, dihydroxydiacetoxy scirpenol at $R_F = 0.32$ and HT-2 toxin at $R_F = 0.31$.

Other trichothecenes of the nivalenol group do not form fluorescent derivatives with sulfuric acid but instead give a dark pink to brown spot when the plate is examined under visible light. A more useful detection procedure for these compounds is spraying with 4-(*p*-nitrobenzyl)-pyridine (NBP: 1% in chloroform), heating for 30 min at 150°C and spraying with tetraethylenepentamine (TEPA). Under visible light a plate developed with chloroform–methanol (9 : 1 v/v) will have blue spots of fusaranon-X at $R_F = 0.36$, and dihydronivalenol at $R_F = 0.07$. A plate developed with benzene–acetone (1 : 1 v/v) will have tetraacetylnivalenol at $R_F = 0.62$, crotocin at $R_F = 0.59$, dihydronivalenol at $R_F = 0.07$ and nivalenol at $R_F = 0.09$.

Another type of trichothecene is a series of macrocyclic di- and trilactonic derivatives of verrucarol. These have been implicated in a disease of horses and other farm animals called stachybotryotoxicosis. Recently they are suspected of contributing to the death of some infants exposed to the air in mouldy houses. Since they contain an ultraviolet-absorbing functional group, they can be detected by using silica gel plates which fluoresce under 254 nm ultraviolet light. The satratoxins appear as dark spots on a white background. If developed with chloroform–2-propanol (99 : 1 v/v) roridin E will appear at $R_F = 0.85$, verrucarin J at $R_F = 0.45$, satratoxin F at $R_F = 0.40$, satratoxin G at $R_F = 0.20$ and satratoxin H (Figure 3) at $R_F = 0.15$.

Zearalenone

Zearalenone (**Figure 3**) is a metabolite of the mould *F. graminearum* also called by its perfect name *Gibberella zeae*. Zearalenone is found in barley, wheat and corn and causes hyperoestrogenism in swine, resulting in infertility and spontaneous abortions. It

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sometimes co-occurs with deoxynivalenol. Zearalenone can be extracted from grain with chloroform and cleaned up using a silica gel column, followed by defatting by partitioning between hexane and acetonitrile. For TLC the samples and standards are dissolved in benzene and spotted on a silica gel and developed with ethanol–chloroform (5 : 95 v/v) or acetic acid–benzene (5 : 95 v/v). Under 254 nm ultraviolet light, zearalenone appears as a greenish-blue fluorescent spot at $R_F = 0.5$. If the plate is sprayed with an aluminium chloride solution and heated for 5 min at 130°C, zearalenone will appear under 365 nm ultraviolet light as a blue fluorescent spot.

Summary

TLC methods have been developed to analyse for a variety of mycotoxins in the commodities in which they occur. TLC densitometric determinations provide precise quantitative data at $ng\,g^{-1}$ to $\mu g\,g^{-1}$ levels. The major advantages of TLC over LC are its low cost and its use as a screening tool. The commercial availability of precoated TLC plates, including silica gel, reversed-phase and high performance plates has resulted in expanded applications in the mycotoxin field. The role of TLC in the analysis of mycotoxins will continue for the foreseeable future.

See also: **II/Chromatography: Thin-Layer (Planar):** Historical Development; Preparative Thin-Layer (Planar) Chromatography. **III/Aflatoxins and Mycotoxins:** Chromatography. **Immunoaffinity Extraction.**

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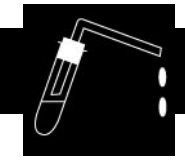
AIR LIQUEFACTION: DISTILLATION



R. Agrawal and D. M. Herron, Air Products and Chemicals, Hamilton Boulevard, Allentown, PA, USA

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Oxygen, nitrogen and argon, the major components of air, have been separated by distillation at cryogenic temperatures for nearly a century. Air was commercially liquefied as early as 1895 by Carl von Linde and also by William Hampson. Linde separated oxygen from air by distillation in a single column in 1902. A commercial plant producing pure nitrogen was already in operation by 1904. The first double-column distillation system, the predecessor to current double-column processes, was commissioned in 1910 by Linde. Argon was produced on an industrial scale by 1913. Today the major industrial companies supplying products from air distillation and liquefaction and also the equipment for this purpose are: AGA, Air Liquide, Air Products and Chemicals, the BOC Group, Linde, Messer Group, Nippon Sanso and Praxair.

The composition of dry and impurities-free air is given in **Table 1**. The critical temperature and normal boiling point (at 101.3 kPa) for each component is also listed. In this table, and in the rest of this chapter, concentration in p.p.m. refers to parts per million on a volume basis. The gases listed in Table 1 are used in